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Gender Difference in Smoking Effect on Chromosome Sensitivity to Gamma Radiation in a Healthy Population

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In the general population, there is variation in radiosensitivity associated with cancer risk. However, data on the role of epigenetic factors in the variation of radiosensitivity are scarce. Thus we investigated the effects of smoking and age on the radiosensitivity of human lymphocytes by measuring the frequency of chromosome aberrations after *in vitro* exposure to γ rays in peripheral lymphocytes from 441 healthy subjects (18–95 years old). We analyzed the frequency of both spontaneous (baseline) and *in vitro* γ-ray-induced (1.5 Gy) chromatid breaks in 50 well-spread metaphases per subject. The overall mean frequencies of spontaneous and induced breaks were 0.02 and 0.45 per cell, respectively. The mean frequency of induced breaks was significantly higher in men than in women ($P = 0.03$) but did not differ by age or ethnicity. Donors who had ever smoked showed a small but significantly increased frequency of induced breaks (mean = 0.47) compared to nonsmokers (mean = 0.41; $P = 0.005$). Further stratification and multivariate analyses revealed that the smoking effect was more pronounced in men than in women. These findings support a smoking effect on radiosensitivity in a healthy population, particularly in men. Therefore, when evaluating the association between radiosensitivity and susceptibility to smoking-related cancers, the effect of smoking should be taken into account. **© 2000 by Radiation Research Society**

INTRODUCTION

Ionizing radiation, including X rays and γ rays, can induce mutations and cell transformation predominantly by causing single-strand and double-strand DNA breaks and thus can lead to chromosomal instability and carcinogenesis (*1*, *2*). Prospective analysis has shown that the frequency of spontaneous (i.e. baseline) chromosome aberrations in lymphocytes is a significant predictor of subsequent cancer risk (*3*). Chromosomal aberrations have been used as a marker for environmental exposure to ionizing radiation (*4*) and can therefore be used to assess radiosensitivity.

Radiosensitivity is a trait of some inherited diseases, including ataxia telangiectasia (AT) (*5*). Radiosensitivity may also be influenced by epigenetic factors such as age, because of the age-related decline in DNA repair capacity (*6*, *7*), cigarette smoking, because of direct damage to chromatin (*8*, *9*), and physical conditions such as pregnancy, because of hormonal effects (*10*, *11*). Intrinsic radiosensitivity has clinical implications, because it may limit the therapeutic dose of radiation and may serve as a predictor of the responses of patients to radiotherapy (*12*). However, the range of variation in and the effect of epigenetic factors on genetically determined radiosensitivity in the general population are largely unknown. This information is also critical for evaluation of gene–environment interactions and the role of radiosensitivity in carcinogenesis in humans in molecular epidemiology studies (*13*).

Sanford *et al.* (*14*) used an *in vitro* G_2-phase radiosensitivity assay that quantifies chromosomal aberrations in the G_2 phase of cell cycle after X irradiation to investigate genetic predisposition to cancers and found that individuals exhibiting deficient repair of DNA strand breaks also had increased radiosensitivity. We previously described an association between high sensitivity to γ radiation and increased risk of developing gliomas (*15*) by using a modified mutagen sensitivity protocol (*16*). In the present study, we further investigated the types and frequency of chromosomal aberrations induced *in vitro* by γ radiation in 441 normal healthy subjects. We tested our hypothesis using previously reported effects of age and smoking on γ-ray-induced chromosomal aberrations.

MATERIALS AND METHODS

Cell Lines and Cell Culture

Lymphoblastoid cells were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ) of the National Institute for General Medical Sciences. To establish the dose–response curve, we used cells of two hyper-radiosensitive AT lines (GM01525C, denoted as AT-1, and GM01526C, AT-2), and two apparently normal lines (GM00131A and

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GM00892B). The AT cells were chosen because their increased radiosensitivity has been demonstrated consistently (*17*) and the heterozygotes or AT carriers in the general population also have increased radiosensitivity that is associated with increased risk of cancer (*18, 19*). The cells were grown in suspension at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 15% fetal bovine serum (GIBCO, Grand Island, NY) without antibiotics.

Study Subjects

The healthy subjects were participants (normal controls) accrued from several ongoing case–control studies in the Department of Epidemiology at The University of Texas M. D. Anderson Cancer Center and included participants in a local health maintenance organization and individuals participating in blood drives in the metropolitan Houston area (*15, 20*). Each participant donated 10 ml of blood, which was collected by venipuncture into heparinized Vacutainers (Becton Dickinson, Inc., Franklin Lakes, NJ). They also completed a short questionnaire eliciting information about demographic and lifestyle factors including age, sex, ethnicity, and use of tobacco, alcohol and supplemental vitamins. Between June 1994 and December 1998, blood samples were collected from a total of 441 apparently healthy subjects. However, in a subset of individuals we obtained completed information on pack-years of smoking from 341 subjects (77.3%) and on supplemental vitamin use from 343 subjects (77.8%).

Mutagen Sensitivity Assay

Chromosome sensitivity to γ radiation was measured by a modification (*21*) of the mutagen sensitivity assay described by Hsu and others (*16, 22*) using bleomycin as the mutagen. In this assay, the mutagen sensitivity was expressed as the frequency of γ-ray-induced chromatid breaks measured 5 h after one exposure to 1.5 Gy incident γ radiation. Briefly, two parallel short-term cultures of each blood sample were established in RPMI 1640 medium supplemented with 20% fetal bovine serum with a final concentration of 112.5 μg/ml phytohemagglutinin (Murex Biotech Limited, Dartford, England) to stimulate growth of T lymphocytes. A ^{137}Cs irradiator (Mark 1, Model 30; J. L. Shepherd and Associates, Glendale, CA) was used for γ irradiation. For each of the cultures, 1 ml of blood was mixed with 9 ml of blood culture medium in a T-25 flask which was then put vertically in the incubator, which was fully humidified with 5% CO_2. The cultures were incubated at 37°C without CO_2 (the flask cap was tightened) for 67 h before irradiation and were shaken gently once every 24 h to resuspend cells. At the end of the incubation, one of the two cultures was irradiated with 1.5 Gy incident γ rays at a dose rate of 14.67 Gy/min for 6.2 s, incubated for an additional 4 h, and then treated with a final concentration of 0.06 μg/ml Colcemid (GIBCO BRL) to induce mitotic arrest. After 1 h, the cells were harvested by conventional chromosome harvesting procedures: The cells were treated for 15 min with 60 m*M* KCl hypotonic solution and fixed for 15 min with freshly prepared methanol:acetic acid (3:1 v:v), followed by preparation of air-dried slides as described previously (*21*). The slides were then stained with 4% Giemsa (Biomedical Specialties, Santa Monica, CA) for 7 min. Each slide was evaluated for chromosomal aberrations using a Nikon Labphoto-2 photomicroscope (Nikon Inc., Instrument Group, Melville, NY). The number of simple chromatid breaks was scored from 50 well-spread metaphases for both treated and untreated samples from each subject, because Lee *et al.* (*22*) have shown that the statistical efficiency reading of 50 and 100 metaphases is similar. The criteria of Hsu *et al.* (*16*) were used to record the aberrations: A chromatid break was scored as one break, and each isochromatid break set and each exchange figure (or interstitial deletion) were scored as two breaks. Gaps were not included in the analyses.

Statistical Analysis

Because over 95% of the aberrations were simple chromatid breaks, the analyses focused exclusively on the frequency of spontaneous (untreated) and induced chromatid breaks per cell. The numbers of breaks per cell were analyzed as a continuous variable. Student's *t* test was used for the comparison of the mean breaks per cell between groups. Because the data on breaks per cell were not exactly normally distributed, we also performed Student's *t* tests on log-transformed data. Multivariate analyses were performed to assess the association between the breaks per cell and other variables of interest. We also applied the negative binomial model, an extension of the Poisson model (*23*), to the data on breaks per cell as a function of age, sex, ethnicity, alcohol use, and smoking status or pack-years to evaluate the deviance residuals. Ever-users of tobacco or alcohol were defined as those who, at any point during their lifetime, had smoked more than 100 cigarettes or had consumed one alcoholic drink per week, respectively. Among ever-users, those who quit smoking (drinking) for more than 1 year were defined as former smokers (drinkers) and the remaining were current smokers (drinkers). Use of any supplemental vitamins in the last 6 months was categorized as ''yes'' or ''no''. All the statistical analyses were performed using Statistical Analysis System software (Version 6.1, SAS Institute, Inc., Cary, NC) and S-Plus software (Version 4, Mathsoft, Inc., Seattle, WA).

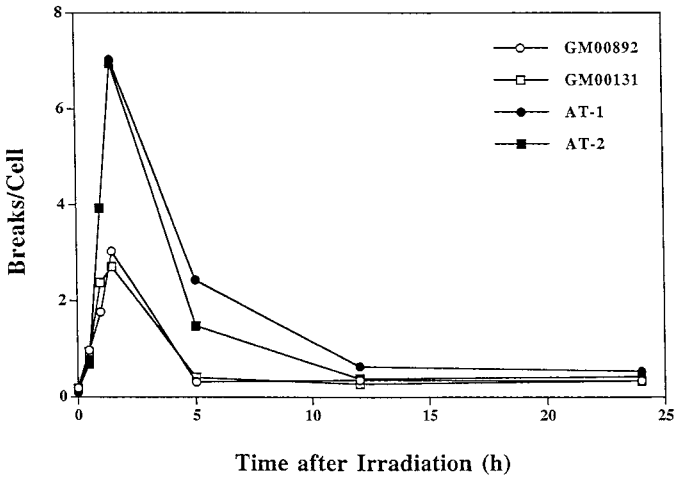

FIG. 1. Time–response curve of γ-radiation-induced chromosomal aberrations in AT and normal cells. The data were derived from one of two independent experiments. The frequency of chromosomal aberrations peaked at 1.5 h after irradiation and decreased to a low level in normal cells but not in AT cells within 5 h.

RESULTS

As shown in Fig. 1, after a γ-ray dose of 1.5 Gy, the frequency of chromosomal aberrations increases with time, reaching a peak at 1.5 h for both AT and normal cells. Five hours after irradiation, the frequency of chromosomal aberrations was reduced to a low level in the normal cells but was still high in AT cells. However, the percentage change in the aberration frequencies between AT and normal cells at 5 h after irradiation was not significantly different, suggesting that although they are sensitive to γ radiation, AT cells may have sufficient capacity to repair the damage induced by 1.5 Gy γ rays. These results are consistent with a reported effect of ionizing radiation on chromosomes of normal human lymphocytes (*24*). Although the differences between AT and normal cells at 1.5 h were significant, such a short time presents difficulties in irradiating and harvesting cells from many samples during the same interval. It

TABLE 1
Univariate Analysis of Chromosome Radiosensitivity by Selected Variables in 441 Healthy Subjects

Variable	No. (%) of subjects	Breaks per cell (mean ± SD)[a]		*P* value[b]
		Baseline	γ-ray-induced	
All	441 (100)	0.02 ± 0.02	0.45 ± 0.23	
Age (in years)				
<40	105 (24)	0.02 ± 0.01	0.46 ± 0.20	Reference
40–49	83 (19)	0.02 ± 0.02	0.45 ± 0.36	0.809
50–59	100 (23)	0.02 ± 0.02	0.46 ± 0.21	1.000
60–69	108 (24)	0.02 ± 0.03	0.43 ± 0.19	0.263
≥70	45 (10)	0.02 ± 0.02	0.44 ± 0.17	0.559
Sex				
Male	266 (60)	0.02 ± 0.02	0.47 ± 0.26	Reference
Female	175 (40)	0.02 ± 0.02	0.42 ± 0.18	0.026
Ethnicity				
Non-Hispanic white	324 (73)	0.02 ± 0.02	0.44 ± 0.24	Reference
Asian	7 (2)	0.03 ± 0.02	0.47 ± 0.30	0.745
African-American	32 (7)	0.02 ± 0.03	0.44 ± 0.20	1.000
Mexican-American	78 (18)	0.02 ± 0.02	0.47 ± 0.24	0.322
Smoking status				
Never	211 (48)	0.02 ± 0.02	0.42 ± 0.18	Reference
Ever	230 (52)	0.03 ± 0.03	0.48 ± 0.27	0.005
Alcohol use				
Never	196 (44)	0.02 ± 0.02	0.44 ± 0.24	Reference
Ever	245 (56)	0.02 ± 0.02	0.46 ± 0.23	0.495
Supplemental vitamin use[c]				
Never	127 (37)	0.02 ± 0.02	0.46 ± 0.23	Reference
Ever	216 (63)	0.02 ± 0.02	0.44 ± 0.24	0.495

[a] Breaks/cell, induced by 1.5 Gy γ radiation.
[b] Two-sided *t* test for γ-ray-induced breaks per cell only.
[c] Only 343 provided information on supplemental vitamin use.

appeared that the experimental scheme of using a γ-ray dose of 1.5 Gy and a time of 5 h after irradiation, which allows cells to repair damage, might differentiate sensitive cells from normal cells. Therefore, we selected this experimental design for the population study.

The distribution by the age, sex, ethnicity, smoking and alcohol use of the 441 healthy subjects is presented in Table 1. The age of the subjects ranged from 18 to 95 years; 105 (24%) were less than 40 years old, 83 (19%) between 40 and 49, 100 (23%) between 50 and 59, 108 (24%) between 60 and 69, and 45 (10%) over 69 years old. There were 266 males (60%) and 175 females (40%). Males (mean ± SD 53 ± 15 years) were older than females (50 ± 15 years) ($P < 0.05$). There were 324 non-Hispanic whites (73%), 78 Mexican-Americans (18%), 32 African-Americans (7%), and 7 Asians (2%). About half of the subjects were ever-smokers (52%) or ever-drinkers (56%). The proportion of ever-smokers was relatively high due to the fact that 125 of the healthy controls included were from an ongoing lung cancer case–control study in which the subjects were matched on smoking status (*20*).

Univariate analysis revealed that the median values of spontaneous and induced breaks per cell were 0.02 (ranging from 0.00–0.20) and 0.40 (ranging from 0.06–3.08), respectively, whereas the overall mean number of spontaneous and induced breaks per cell was 0.02 and 0.45, respectively (Table 1). Although the mean was greater than the median, the results for both transformed and untransformed data are similar (data not shown), and we therefore presented the results for the untransformed data only. In general, the mean of induced breaks per cell was more than 10-fold higher that that of spontaneous breaks per cell (Fig. 2). Because the frequency of spontaneous breaks per cell was very low and negligible compared to the induced breaks per cell, only induced breaks per cell were used for statistical comparisons as recommended (*16*). Aside from one subject whose break frequency was 3.08, induced frequencies ranged from 0.06 to 1.20, representing a 20-fold variation in this healthy population. The relatively large variation observed is partly due to individual variation and is because only 50 metaphases were evaluated. The variation would be smaller had more metaphases been evaluated.

More than 95% of the induced chromosome aberrations were simple chromatid breaks; the rest were isochromatid breaks or exchange figures (data not shown). The mean number of induced breaks per cell was significantly higher in men than in women ($P = 0.026$) but did not differ by age or ethnicity (Table 1). Because there was no difference

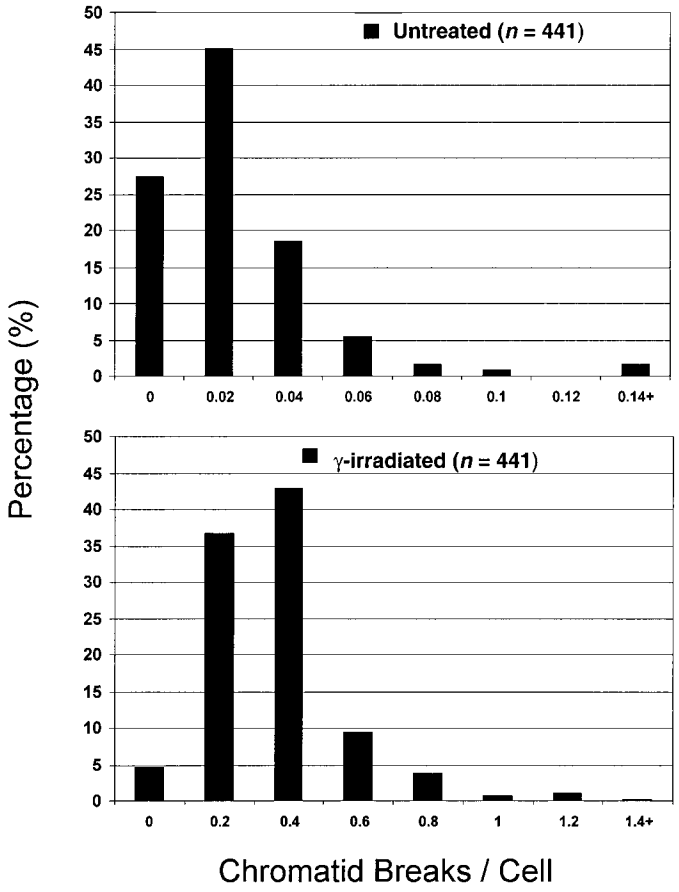

FIG. 2. Percentage distribution of spontaneous and γ-ray-induced chromatid breaks in 441 healthy subjects. The mean and median spontaneous breaks per cell were 0.02 and 0.02, respectively, and the mean and median γ-ray-induced breaks per cell were 0.45 and 0.42, respectively.

in current and former smoking (or drinking) status, these two variables were recoded as ever (former and current) and never-user groups. While alcohol use did not have any effect on the number of induced breaks per cell, we observed significantly increased induced breaks per cell in ever-smokers compared to never-smokers ($P = 0.005$).

The sex distribution by selected variables is presented in Table 2. In general, males were older ($P = 0.010$) and more likely to be alcohol drinkers ($P = 0.001$) and less likely to use supplemental vitamins ($P = 0.001$) than females. Further correlation analysis (Table 3) revealed that age was positively correlated with smoking status ($P = 0.004$), pack-years ($P = 0.001$), alcohol use ($P = 0.003$), and baseline number of breaks per cell ($P = 0.028$), but not with γ-ray-induced breaks per cell ($P = 0.278$). Females were younger ($P = 0.033$) and more likely to take supplemental vitamins ($P = 0.048$) (Table 3). Men had significantly higher levels of γ-ray-induced breaks per cell ($P = 0.027$) but not baseline breaks per cell ($P = 0.205$). Smoking status and greater number of pack-years, but not alcohol use, were both correlated with baseline breaks per cell ($P = 0.008$ and $P = 0.005$, respectively), whereas only smoking status

TABLE 2
Distribution of Selected Variables between Males and Females

	Males		Females		
Variable	No.	(%)	No.	(%)	*P* value[a]
All	266	(100)	175	(100)	
Age (in years)					
<40	58	(22)	50	(29)	0.010
40–49	50	(19)	30	(17)	
50–59	50	(19)	50	(29)	
60–69	76	(28)	32	(18)	
≥70	32	(12)	13	(7)	
Smoking status					
Never	119	(45)	92	(53)	0.107
Ever	147	(55)	83	(47)	
Alcohol use					
Never	101	(38)	95	(54)	0.001
Ever	165	(62)	80	(46)	
Supplemental vitamin use[b]					
Never	91	(47)	36	(24)	0.001
Ever	101	(53)	115	(76)	

[a] Two-sided χ^2 test.
[b] Only 343 provided information on supplemental vitamin use.

was correlated with γ-ray-induced breaks per cell ($P = 0.006$). Interestingly, there was no correlation between the baseline and γ-ray-induced breaks per cell, which suggests that these two sets of measurements may be independent.

To further evaluate the effect of the selected factors on γ-ray-induced breaks per cell (using the number of breaks in 50 metaphases) in multivariate analysis, we used the negative binomial model, because our experience in analyzing this type of data with an overdispersion suggests that it can generate the model with the best fit (*25*). As shown in Table 4, smoking status remained a significant and independent predictor for induced breaks per cell in a model including age, sex, ethnicity, smoking status, and alcohol use. When we further incorporated the data on pack-years (from a subset of 341 subjects) and/or supplemental vitamin use (from a subset of 343 subjects) into the model with smoking status, the model remained virtually the same (data not shown), suggesting that both the pack-years and supplemental vitamin use were not predictors for induced breaks per cell in the presence of the variable ''sex''. Further, removing the smoking status variable in the analysis did not cause a substantial change in the coefficients of the remaining variables (data not shown), which indicates that number of pack-years is independent of smoking status. Although no interaction terms were statistically significant (data not shown), we observed different slopes for males and females ($P = 0.005$) and for smoking status ($P < 0.001$) (Table 4). Therefore, we fit the model again for males and females separately. As shown in Table 4, in males both smoking status and alcohol use were significant (positive) predictors for the induced breaks per cell, where-

TABLE 3
Correlation between Chromosome Radiosensitivity and Selected Variables in 441 Healthy Subjects

	Correlation coefficient/*P* value						
Variables[a]	Sex	Smoking	Pack-years	Alcohol	Baseline mutagen sensitivity	Gamma-ray induced mutagen sensitivity	Supplemental vitamin intake[b] (0, 1)
Age (in years)	−0.101	0.138	0.262	0.142	0.110	0.052	−0.107
	0.033	0.004	0.001	0.003	0.028	0.278	0.048
Sex (1, 2)		−0.077	−0.024	−0.161	−0.060	−0.106	0.106
		0.108	0.655	0.001	0.205	0.027	0.027
Smoking status (0, 1)			0.650	0.157	0.126	0.133	−0.065
			0.001	0.001	0.008	0.005	0.227
Pack-years (in years)				0.172	0.010	0.007	−0.085
				0.001	0.006	0.893	0.151
Alcohol use (0, 1)					0.030	−0.033	0.047
					0.530	0.495	0.383
Baseline mutagen sensitivity (breaks per cell)						0.078	−0.017
						0.100	0.758
Gamma-ray-induced mutagen sensitivity (breaks per cell)							−0.084
							0.119

[a] Sex: 1 = males, 2 = females. Smoking status, alcohol use, and supplemental vitamin use: 0 = never, 1 = ever.
[b] Only 341 and 343 subjects provided information on pack-years and supplemental vitamin use, respectively.

as in females only age was a significant (negative) predictor for induced breaks per cell after controlling for both smoking status and alcohol use. This gender difference was further illustrated by plotting the deviance residuals as a function of fitted values: Age was not a significant predictor for the fitted values in both males and females (data not shown), and smoking status was a significant predictor for the fitted values in males but not in females (Fig. 3).

TABLE 4
Regression Analysis of Induced Chromosomal Aberrations by Negative Binomial Model for the Full Data Set with Smoking Status and for Males and Females Separately

	Fitted values		
Model	Coefficients	SE[a]	*P* value
All subjects (n = 441; deviance = 440.57; df = 435; dispersion = 1.01)			
Intercept	3.238	0.082	<0.001
Sex	0.061	0.021	0.005
Age	−0.003	0.001	0.016
Ethnicity	−0.008	0.024	0.742
Smoking status	0.149	0.042	<0.001
Alcohol intake	0.082	0.043	0.057
Males only (n = 266; deviance = 264.61; df = 261; dispersion = 1.01)			
Intercept	3.172	0.123	<0.001
Age	−0.002	0.002	0.239
Ethnicity	0.051	0.032	0.401
Smoking status	0.026	0.057	<0.001
Alcohol intake	0.118	0.057	0.038
Females only (n = 175; deviance = 177.02; df = 170; dispersion = 1.04)			
Intercept	3.299	0.122	<0.001
Age	−0.005	0.002	0.022
Ethnicity	−0.027	0.037	0.454
Smoking status	0.041	0.063	0.523
Alcohol intake	0.036	0.065	0.583

[a] Standard error.

DISCUSSION

It is estimated that approximately 10% of the population may be radiosensitive (*26*). However, few studies have investigated the effect of epigenetic factors such as cigarette smoking on radiosensitivity in the general population. In this study, we showed that in 441 healthy subjects, cigarette smoking increased radiosensitivity as measured by γ-ray-induced chromosomal aberrations. Although age correlated with an increased frequency of spontaneous chromosomal aberrations, this age effect was largely explained by the effect of smoking. The observed sex difference was also largely explained by the effect of smoking. Although it remains unclear why smoking has a differential effect on radiosensitivity in males and females in this study population, the findings strongly suggest that smoking should be considered a confounding factor in designing future population studies of radiosensitivity when smoking is also associated with the disease outcome of interest.

Although a recent study that used chromosomal painting for chromosomes 1, 2 and 4 found a positive association between increased age and increased frequency of stable aberrations such as translocations and insertions in Chernobyl cleanup workers (*27*), we did not find a significant age effect on spontaneous chromatid breaks. However, a smoking effect on spontaneous chromatid breaks was found, and this effect was correlated with age. This finding is consistent with another large human population study, in which no significant relationship between age and the frequency of baseline (or spontaneous) chromosomal aberra-

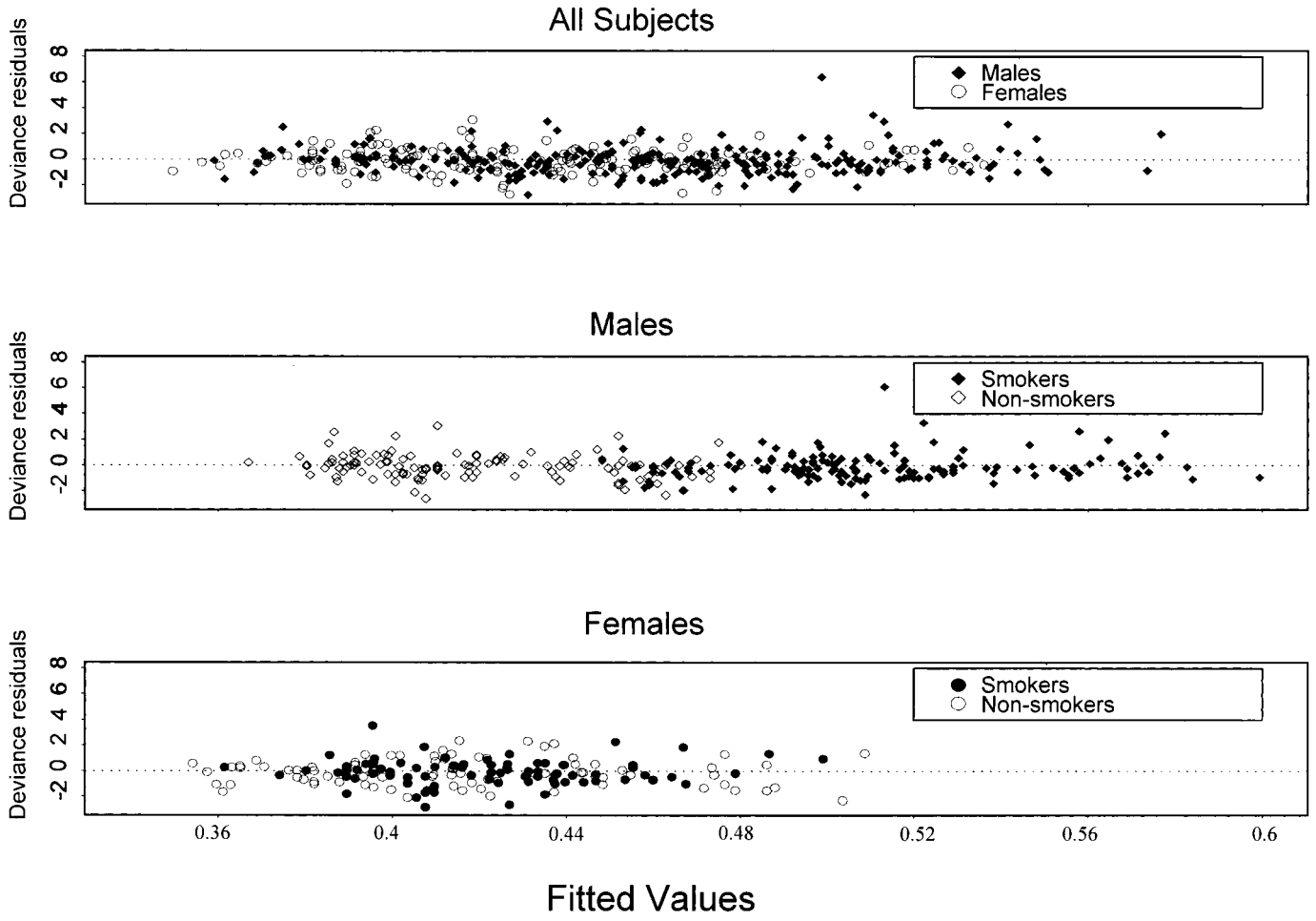

FIG. 3. Plot of the smoking effect by the deviance residuals (*y* axis) as a function of the fitted values (*x* axis) (the number of breaks in 50 metaphases) obtained using the negative binomial model. The top panel is for all subjects ($n = 441$) by sex. The middle panel is for males ($n = 266$) by smoking status (ever, never). The bottom figure is for females ($n = 175$) by smoking status (ever, never). It is clear that smoking effect contributed most to the variation in the observed frequencies of chromosome aberrations induced by γ radiation.

tions was observed with the exception of dicentric chromosomes, the latter being explained by the effect of cigarette smoking (*28*). Our finding of a smoking effect on baseline chromosomal aberrations is also consistent with the results of several other published studies. For example, it has been shown that smoking causes chromosomal aberrations in healthy subjects (*29, 30*). Slozina *et al.* (*31*) reported that 6–10 years after the Chernobyl accident there was an increased frequency of chromosomal aberrations, particularly chromatid exchanges, in lymphocytes of 60 liquidators who were smokers compared to 39 liquidators who were nonsmokers. Using chromosomal painting for chromosomes 1, 2 and 4, another study reported a significant association between smoking and the frequency of stable aberrations. Newborns whose mothers smoked during pregnancy had a 1.5-fold increase in such stable aberrations (*32*). Kao-Shan and colleagues (*33*) reported a dose-dependent increase in smoking-induced sister chromatid exchange in 18 smokers with a median age of 25 years, and the frequency increased as years of smoking increased. Therefore, it is possible that smoking and ionizing radiation have a synergistic effect on chromosomal aberrations. However, Ban *et al.* (*34*) reported that in 937 atomic bomb survivors, radiation and cigarette smoking had no significant effect on background and X-ray-induced micronucleus frequencies. Bigatti *et al.* (*35*) reported that the frequency of chromosomal aberrations was increased in 63 hospital workers who were exposed to low levels of radiation during diagnostic procedures regardless of smoking status. In a study of 135 nuclear power plant workers and 135 age-matched controls, Chung *et al.* (*36*) found a significantly increased frequency of chromosomal aberrations in the exposed group but did not find any association between spontaneous chromosome aberrations and smoking or alcohol consumption.

Because most studies mentioned above were cross-sectional, it is likely that differences in study designs and sample sizes may have contributed to the observed discrepancies. For example, one of the major factors in these studies is the temporal relationship between smoking and radiation exposure, which is often difficult to assess in such population studies. The *in vitro* γ-radiation-induced mutagen

sensitivity assay reported here provides an opportunity to investigate whether prior exposures such as smoking can sensitize the cells to radiation exposure. Our data suggest that such a radiation sensitization effect appeared to be stronger in males than in females.

The gender difference in the effect of smoking on sensitivity to radiation observed in this study is intriguing. The mutagenicity of cigarette smoke has been investigated intensively in males. Studies have shown that smoking causes a decrease in seminal plasma antioxidant levels and an increase in oxidative damage to sperm DNA (*37*), which results in overall reduction in semen quality in terms of sperm count and motility and increases the level of abnormal cells including aneuploidy in sperm (*38*). Although the effect of smoking may be reduced by supplemental vitamin use in females as suggested in the univariate analysis, this effect was not substantiated in the multivariate analysis. Therefore, the absence of a smoking effect in females in this study is perplexing and warrants further investigation. Although sex hormones may influence the metabolism of tobacco carcinogens (*39, 40*), the influence of other smoking-related behaviors such as inhalation when smoking or using different brands needs to be explored.

Radiosensitivity of lymphocytes in G_2 phase has also been suggested as a useful biomarker for identifying individuals at high risk of developing cancer of the breast (*41*), brain (*15*), and skin (*42, 43*), as well as in individuals with a genetic predisposition to cancer (*44*). This may be because of an inherited DNA repair deficiency, as has been seen, for example, in patients with xeroderma pigmentosum (*45*). Measuring radiosensitivity in peripheral blood lymphocytes is a convenient, feasible assay for population-based studies of cancer susceptibility (*15*). However, the use of lymphocytes has limitations in terms of interpretation and extrapolation to other tissues. For instance, Geara *et al.* (*46*) found no correlation between radiosensitivity of lymphocytes and of skin fibroblasts from 25 subjects.

It has been suggested that increased chromosomal instability may be associated with poor DNA repair capacity (*23*). Badie *et al.* (*47*) reported hyper-radiosensitivity and poor DNA repair in fibroblasts from a radiosensitive leukemia patient, but this link between radiosensitivity and DNA repair is not well established, even in radiosensitive AT patients (*5*). Although an age-related decline in DNA repair capacity in the general population has been reported (*7*), we did not observe an age-related increase in the frequency of induced chromosome aberrations that is independent of smoking in this study population. This is consistent with a report of no association between spontaneous chromosome aberrations and age in nuclear power plant workers (*40*) and atomic bomb survivors (*34*).

In conclusion, although we observed a significant effect of smoking on radiosensitivity in a healthy population, the results in this study should be interpreted with caution and need to be verified in further studies with detailed information on smoking and occupational exposure. Nevertheless, these results strongly suggest that cigarette smoking should be considered as a confounder in future case–control studies on the role of radiosensitivity in the etiology of smoking-related cancers.

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